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THE INHERITANCE OF COLOR IN SHORT HORN CATTLE

II.

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In order to adduce further evidence bearing upon the problem of the inheritance of color in cattle the following observations on the occurrence of dominant and recessive whites in other animals are reported. Many species of animals have some strains with solid white coats and others with coats made up of both white and pigmented areas. The white in such latter coats is always possessed in a somewhat definitely arranged and progressive system of areas characteristic of each species, spreading from the first of these areas until the entire body is covered. Thus in the guinea pig the whitening process begins with the underline and large "centripetal" body blotches and spreads until the hair, skin and body pigments are entirely removed, the eye and the "centrifugal" coat pigments persisting the longest. In the domestic cat the process begins with the anterior underline and collar, from which areas it spreads in large blotches. The rabbit's pigment areas behave similarly. With the particolored dog of any or no breed, the whitening process begins with a white line down the middle of the face, a

white chest, a white collar and generally a white tail tip. With cattle of all breeds and crosses possessing broken color patterns the process begins with a white belt at the rear flank, continues with another at the fore flank, a white underline and a white forehead. With horses of the English breeds it begins with a blaze face and white feet, continuing with large body blotches. With the French and the desert breeds it seems to begin with a "dappling" of the body hair—dark pigment persisting longest on the legs—and continues through a lighter "dappling" to white, the skin remaining black, while the sharper whitening process seems to follow the sequence observed in English breeds. In the case of the piebald negro the median face line is quite noticeable. Thus, while there is for each species a characteristic pattern, there is in reality a somewhat common pattern in all species of mammals possessing particolored individuals. This common pattern is described as follows: White line down the face, white underline, white anterior belt or collar, white rear flank belt and white feet and switch. These white areas may, in animals possessing but little white, be represented by several smaller areas, but always near the median line of the areas white in the larger pattern, which smaller areas may fuse as the pattern becomes coarser. Thus the white nose and forehead in some cattle may in others make a continuous white line down the face. The white spreads from the areas just defined with much the same sequence as a fire would spread over a "hide-shaped" meadow, starting at the centers homologous to the first white areas of the coat. In all cases the pigments seem to persist the longest in and about the eyes and ears and at the buttock—the "centrifugal areas" mentioned by Castle.

The coat of a white Shorthorn may consist of: (1) Solid dominant white covering red (quite rare); (2) some definite coat areas dominant white covering red, others albinic white (very common); (3) some areas albinic white, others dominant white not covering red

(quite common). In eye color the white Shorthorn is either blue or brown; the roan and red Shorthorns are always brown eyed. The characteristic pigment areas may, in domestic animals, become subjected to rigid selection, resulting in modified forms—as the white belt of the Dutch belted cattle and the white head, neck and underline of the Hereford. Approximations to the former and to the reciprocal of the latter modifications (*not* of the species pattern) are commonly observed among Shorthorns. In general, however, color patterns are quite characteristic of the species and are quite persistent. Greatly modified patterns are seldom seen and, moreover, the reciprocal coloration is *never* seen.

Not only does the whitening process begin at definite centers quite specific for each species, but it also seems to be definitely progressive in tissues carrying pigment. Thus, the whitening process begins in man with the skin, extending to the hair, the iris, and finally the choroid. Partial albinos often have blue eyes—the absence of the iris pigment but the presence of the choroid. In the guinea pig the hair and skin pigments generally seem to disappear before those of the eye. Castle[16] reports a guinea pig with an area of red hair underlaid by a patch of black skin, and observes that a white dog may have a patch of pigmented skin somewhere under the hair coat. Similar phenomena are found in all species having particolored strains. It is a matter of common observation that a white patch of skin or hair unsymmetrically covering but one eye of a dog or horse sometimes gives this animal a "glass eye," *i. e.*, unsymmetrical eye color, the blue eye being surrounded by white hair and skin while the dark eye is surrounded by dark tissues; this, however, is not always the case, for many times both eyes are dark.

In horses and cattle the hair is first whitened, then the skin, the iris and the choroid follow in the order named. A black horse or cow may have a spot of white hair on some portion of the body; it may be entirely underlaid by

[16] "Heredity of Coat Characters in Guinea-pigs and Rabbits," p. 46.

black skin—this is especially apt to be the case with small spots, or under the center of such an area there may be a pigmentless skin—generally characteristic of the larger areas, while its margin is underlaid by black skin, but *never the reverse*. In some instances, however, the pigmentless skin and hair areas exactly coincide. The hoof of the white foot of a horse or cow is generally white, that of the dark foot always black; however, quite often a white patch or streak will extend to the hoof and then come to an abrupt end, the hoof continuing in a vertical line the same pigment possessed by the skin immediately underneath the lighter hair patch giving rise to the hoof; thus the hoof is as dark or darker, but never lighter, than the hair patch immediately above. In spotted horses it is observed that a white coat spot crossing the mane will sometimes whiten it, while in other instances it will not. Mr. Chas. E. Burns, the pony breeder of Peoria, Ill., writes:

> We naturally expect spotted Shetlands from spotted ancestors but can say that very frequently I have bred a spot to a spot and the offspring has been a solid color. On the other hand, I have very frequently had spotted colts from solid-colored parents. The fact that there is spotted blood in the ancestors of the solid colored ponies accounts perhaps for the spots, and *vice versa*. There seems to be no sure rule in governing the color of a Shetland. The mane and tail are not always the same color as the adjacent color patches of the coat. Very frequently I have seen a white mane come right out of a black patch, although as a general rule the color of a mane is the same color as the adjacent coat of the pony. This is general also as regards the tail, but very frequently, as I say, a black tail comes out of a white spot, or a white tail out of a black spot, and often the tail is both black and white.

Mr. C. R. Clemmons, of Coffeyville, Kans., writes:

> I have been breeding spotted Shetlands for twenty-five years. I find that many mares of a solid color will bring spotted colts quite regularly when bred to a spotted stallion having considerable blood from these colors but every now and then there will be a foal of perfectly plain color as the result of this same mating. I am of the opinion, however, that a spotted color breed could be obtained by breeding in these colors and perhaps inbreeding.

The mane and tail of a spotted Shetland are not always of the same color as the adjoining patches of the coat, there is sometimes a distinct color line between the mane and tail and the coat."

Mr. W. A. Long, of Greeley, Ia., offers the following evidence:

We have no recollection of seeing any roan Belgian stallions with white manes and tails. Our experience with the roan stallions has been that the colts are principally roan. We have in mind one roan stallion that we imported that sired 68 colts one year and they were all roans out of all colors of mares. We handle many chestnut horses with white manes and tails, and they sire principally all chestnut colts, but there will be some bays and other colors, as all colors are represented in the Belgian breed. They do not all sire the white mane and tail but many of the colts are so marked.

Mr. A. W. Hawley, of Pioneer, Ia., says:

I had a beautiful light mane and tail chestnut from a black Belgian mare and a black Percheron stallion.

The roan stallion above referred to doubtless corresponds exactly to either type No. 7 or No. 8 of Shorthorn cattle, the dominant duplex white always persisting and the hairs of the second network remain either red or black, making the familiar "red-roan" or "blue-roan," depending upon the gametic composition of the dam, epistacy and the laws of chance. Belgian horses resemble Shorthorn cattle in that they are a breed of many colors, including the interesting roan. Indeed, the roan, silvered, barred, "agouti," mottled, piebald, flea-bitten and other variegated types of animals of all species so characterized seem to behave in inheritance in a manner typified by the roan Shorthorn.

While the mane and tail are generally of the same color as the adjacent body coat, there is often a pigment differentiation—the coarser hair whitening first. This phenomenon is also exemplified in the case of black or spotted cats, which often have white "mustaches" growing from black or dark skins and coats, but never the reverse. Among wild animals the silver fox and the

silver-tipped bear present instances of the whitening process beginning with the hair tips. Recall, in this connection, the lighter colors sometimes present on the hair tips of the cattle crosses reported by Professor Wentworth, and the "albinic" superficial tissues of the Silkie fowl with its pigmented deeper tissues. Thus it seems that with mammals and with some birds the whitening process begins with the more superficial tissues and continues to the deeper ones; with mammals, coarse hair, fine hair, skin, nail, sclerotic, iris, choroid, being the order followed.

Permit a short digression into the plant kingdom. Of the two or three hundred varieties of the dent corn, all of the yellow and red varieties have red cobs and all of the white varieties have white cobs, with the exception of St. Charles County white, which has a red cob.

Jack-stock breeders in America are making an effort to establish a race of black animals with white points, and the following evidence, while primarily bearing on this problem, is typical of the behavior when not involving the whitening process of pigments of domestic animals. In the *Breeders' Gazette,* May 10, 1911, a breeder states this problem:

> A jack is of good size, well made in every way but he is of maltese color. He is exactly the color of his sire and his sire was a popular jack in his locality and a first-class mule-getter. Is this color a real objection? What is the prevailing color of mules sired by the maltese-colored jacks?

To which L. M. Monsees, of Sedalia, Mo., the jack-stock breeder and authority, responds:

> I have seen some extra good mule jacks of the maltese color. A maltese or blue jack, if from a good, large family of good blood, and himself a good individual, will no doubt prove a good breeder. He should be expected to get good solid colors—bays, blacks, browns, blues and chestnuts.

Thus it seems probable that, when different parental pigments, but not the whitening process, are involved, the

pigments of the offspring are due to either a simple Mendelian mixture of various dilutions of parental pigments with their resultant hypostatic effect, or to minor reaction between the determiners presented by the two parents resulting in modified pigment bodies.

Barrington, Lee and Pearson's study of color in the gray-hound—*Biometrika,* 1904—presents evidence that might well be given such an interpretation. Their elaborate tables measure accurately the correlation of the color of ancestry and offspring in this animal, but they do not explain what takes place in the zygote upon its creation by the union of two somewhat differently organized and differently descended gametes; nor do they clarify the conception of gametic organization. It is, however, primarily a study in the mathematics, not the chemistry, mechanism nor biology of inheritance.

The black mane, tail and feet of the bay and of some blue roan horses, and the white mane, tail and feet of the chestnut Belgian seem to indicate that in horses the whitening process may proceed somewhat out of synchronism in its tissue and area sequences. The white mane and tail seem to be causatively correlated with the chestnut coat of the Belgian, which white seems to be recessive to the heavier pigments. Moreover, when the destroying process attacks highly organized pigment bodies, is the destruction always complete? May there not be resting stages in this destruction and may not the series—blacks, browns, bays, chestnuts, sorrels, duns and creams—besides being different dilutions and hypostatic effects of different pigments, represent these stages? Furthermore, may there not be a pigment sequence as well as an area, tissue and ontogenetic sequence involved in the whitening process? And are the chestnut, sorrel, dun and cream pigments the ones most readily destroyed by the antibody?

Note in this connection that in dogs and other mammals having some individuals with black, tan and white areas, the black areas are quite often bordered by a zone of tan,

and often small tan but rarely small white spots are found within the larger black areas. These are the conditions expected if the tan were an intermediate product resulting from the attack of the destroying antibody upon the determiner for the heavier pigmentation. The sequence of color bands along the hairs of the wild and agouti cavies, viz., heavily pigmented brown tip, yellow band and leaden base, is also suggestive of the same derivation of the yellow.

Besides an area progression and a tissue progression involved in the whitening process in animals, there is also an ontogenetic progression of the same process. In man and in many pigmented animals a progressive grayness, called "senile white," comes with old age, in some strains earlier than in others. White horses—dominant white—are always born pigmented, but soon change to white—juvenile white, it might well be called. White Leghorn fowls are hatched white and, save for a senile deposit of pigment, remain so.

The observed facts seem to demand intra-zygotic inhibition and reaction quite closely approximating the following hypothetical processes: In a germ cell of some heavily pigmented animal, say, of a black Angus bull, let there be a specific chemical determiner (N) for black pigment in the entire skin and hair coat and in the sclerotic, choroid and iris. This determiner reacts like and indeed may be a body closely related to the enzymes, in that both may be weakened, exhausted, or totally inhibited without being impaired or destroyed by the presence of varying amounts of an antibody of some sort, still greater amounts of which set up chemical reaction resulting in partial or total destruction, depending upon the relative quantity and intimacy of the two bodies in much the same manner as trypsin is totally inhibited but not destroyed by .05 per cent. of lactic acid, but is totally destroyed by .1 per cent. of hydrochloric acid.[17] In the germ cell of a white mate of the aforementioned

[17] Green, "The Soluble Ferments and Fermentation," p. 198.

animal let there be an antibody (W) (analogous to the acids in the above illustration) substituted for and placed homologously to the determiner (N) for black pigment, which antibody is capable of weakening, inhibiting and finally of totally destroying the determiner according to the relative quantity and intimacy of the two bodies. Let this antibody (W) exist in a quantity large enough to totally inhibit the ontogenesis of N, but not to effect its destruction. Now let fertilization take place; the F_1 generation is white. A white so behaving is said to be dominant. Because there was only inhibition of N with no chemical reaction between N and W, and segregation may take place in later generations according to the familiar formula, F_1 is said to be simplex in reference to this unit character. If, however, the antibody in quantity sufficient for inhibition makes its intrusion de novo into a gamete possessing a determiner for N and this mutant germ cell meets another of similar origin or descent, and the total amount of the antibody is still sufficient to cause reaction, a duplex dominant white offspring results, which, mated with one of its own kind, will establish a race of white animals, inhibiting somatically in heredity until further disturbance by extraneous intrusion or by hybridizing the determiner N. Animals of this sort upon hybridizing—as Davenport has shown in his white Leghorn crosses—may be made to yield the ancestral coloration. If the antibody exists in quantity sufficiently great to inhibit absolutely all of the determiner, with an excess sufficient to cause chemical reaction destroying a portion of N, then partial albinism results and the offspring, although entirely white, will possess some definite areas of dominant white covering pigment, and others of albinic white, breeding exactly like the white Shorthorns designated in this study as type No. 6. If, however, in the germ-cell of the white mate a still larger quantity of W be present (exactly large enough to effect the total destruction of N) upon fertilization N and

W react and are destroyed, the F_1 generation is white—this time albinic white, mutants. W and N both being destroyed, these animals are nulliplex and breed according to the familiar formula.

As still another alternative, let W exist in still larger quantities and the mating take place; not only is all of N destroyed but there is an excess of W which gives some areas of duplex dominant white not holding the pigmented color as a recessive trait—in quite the same manner as the Shorthorns designated in this study as type No. 9 possess a coat solid white, some areas of which are dominant white not covering the red and the remainder of the areas are albinic white. A still greater amount of W will apparently effect the total destruction of N, making the offspring in the entire coat duplex dominant white, not holding N latent in the gametes and not capable of "reversion."

Let the antibody exist in very small quantity, insufficiently large to inhibit the ontogenesis of N, and let fertilization take place. It is conceivable that the antibody in such small quantity might have the same effect upon N as alcohol has upon an enzyme, in which case N would play its usual part in ontogenesis, but, being constantly harassed by W, would finally be inhibited or destroyed. The F_1 generation would then show senile grayness, as in man; here again the most superficial tissues are first attacked. If the antibody (W) is a trifle more concentrated the F generation will be born pigmented, but will develop juvenile white, as with the white horse, which as previously described is born with pigmented hair and skin—the skin remaining black and the hair turning white. Thus the process seems to be progressive, depending upon different intrusions de novo—"mutations"—and different inheritance lines for the presentation of various quantities of the antibody effecting the destruction of N in a definitely progressive ontogenetic, area and tissue sequence.

This is the hypothetical picture of intra-zygotic reaction

demanded by the somatic behavior in inheritance of coat pigments and patterns in Shorthorn cattle and in the other instances above cited.

Now, let some further observations be reported and then fitted to this conception for its support or rejection.

In the *Breeders' Gazette* (April 12, 1911) in response to an inquiry concerning the behavior in inheritance, with special reference to the possibility of spotted offspring of a white stallion, described as follows:

He is white with pink skin and would be albino but for a very few small specks in the skin and his dark eyes.

Dr. W. E. Castle answers:

The dark-eyed white condition is closely related to the piebald condition. It may indeed be regarded as an extreme variation of the piebald state in which the white spots cover the entire body except the eye. Most black-eyed white animals produce a certain number of piebald offspring, even when bred to animals exactly like themselves.

In reply to a request, W. P. Newell, of Washburn, Ill., the owner of the white stallion, supplies the following data:

The albino offspring of my stallion do not have pink eyes, but have "glass" or "watch" eyes.

Their hoofs are white or flesh color; there are no spots in the skin and not a colored hair on them. Not all of his white colts are albinos, some of them have a few colored hairs in mane or ears; these I do not refer to as albinos.

As a two-year-old this stallion was bred to six mares. Each one of these six produced a white colt.

As a three-year-old he had thirty-nine mares; got thirty-three in foal. About half of these were white, the others solid colors. These mares were very ordinary and of all colors, every size, shape and age. Following are a few of the instances: Bay mare got white colt; bay got black colt; two blacks got white colts; black got black colt; white and buckskin spot got pure albino; dapple gray got white colt; flea-bitten gray got white colt; three or more brown mares got white colts; two or more brown mares got brown colts; brown mare got pure albino; one sorrel got pure albino; one sorrel got brown colt. This will give you an idea of how his colts are colored.

Nothing is given and not much can be deduced con-

cerning the gametic make-up of these brood mares, but this interesting stallion seems to be barely on the dominant white side of the critical border between dominant white and albinic white. Had the whitening factor been a little more concentrated in the zygote giving rise to him, doubtless the ontogenesis of his choroid would have been inhibited or destroyed, the determiner for much of his more superficial pigmentation would have been destroyed and he would have been a *true* albino. Some of his germ cells seem to contain the antibody W in quantity and distribution adequate to inhibiting the quantitatively definite determiner for pigmentation found in some of the gametes of many pigmented mares; others of his gametes seem to lack this specific antibody, having in its place a determiner for dark pigmentation, hence, he is apparently simplex with reference to his dominant white determiners. If one of his gametes possessing W unites with a mare's gamete possessing pigmentation determiners greater than the quantitatively definite determiner above referred to, the inhibition will either not take place or it will take place incompletely—in the latter case resulting in some modification of the solid-color coat and skin condition. If the mare's gamete possessing less of the pigmentation determiners than the optimum quantity above referred to meets one of the stallion's gametes possessing W, the offspring will be white—dominant if the relative concentration of the determiner and the antibody is such as to cause only inhibition; recessive, *i. e.*, "albinic" if reaction occurs.

Let us consider the criteria of albinism. The general conception among investigators and writers on the subject seems to be that all strains of albinos have originated through dropping from the germ-plasm determiners for pigmentation previously possessed, rather than to have descended from ancestral types *never* possessing such pigmentation. Generally an animal is designated as "albino" when inhibition and reaction

have covered the entire skin, hair, nail and eye pigments. Castle[18] in his "Heredity of Coat Characters in Guinea-Pigs and Rabbits," excepts "centrifugal" areas. There is, moreover, no reason to believe that the pink eye of an animal may not result from the inhibition of the pigment determiner as well as from its destruction. In the progressive development of whiteness from senile white, juvenile white, dominant white covering pigment, albinic white, to dominant white not covering pigment, there seems to be, as we have seen, a species of tissue resistance as well as of area progression to this inhibition and reaction; the pigment of the deeper tissues being more generally resistant, or at least slower or later in succumbing to the attacks of the antibody. These deeper tissues, when dark and covered by the pigmentless tissues, give rise to a condition that is proved by experimental breeding generally to be dominant white. This is quite consistent with the present conception, for if the skin below is still pigmented it is quite probable that the hair pigments are only inhibited and not destroyed, and by the time the inhibiting process reaches the choroid, the destroying process is probably quite complete in the hair, and the animal is quite properly designated as an "albino"—recessive white. It must be borne in mind, however, that albinism may be either partial or complete; it may affect the entire coat color or it may affect only a limited area or a specific tissue. In partial albinism the eye is often blue—the absence of superficial pigments but presence of the deeper. Thus albinos become of great interest, and the study of their behavior very complicated, on account of this nascency of mutation. The intricate organization of the gamete can be determined only by the study of its ontogenetic sequence and end which, however, strongly suggest that chemical bodies within the germ cell behave exactly as such bodies within the test tubes of the laboratory. The disturbance of a single determiner may

[18] "Heredity of Coat Characters in Guinea-pigs and Rabbits," p. 9.

cause an accompanying correlation in something like the following manner: Consider the determiner for some definite somatic structure in a germ cell of one parent to be destroyed by an antibody analogously placed in the germ cell of the other parent; this chemical reaction must leave a product, which product it is conceivable may cause considerable havoc in so intricate a mechanism. There is no reason to believe that this product would of necessity confine itself to reactions with determiners first attacked; it might indeed be conceived to disturb or to destroy certain determiners for other tissues and forms.

The Silkie fowl seems to have received a very severe and peculiar upset in its determiners for pigmentation—note its black eyes and black deeply seated body pigments, together with its "albinic" plumage. Neither is there any probability, except by chance, of parallelism or similarity between the mechanical or chemical cause of such reaction and the resulting determiners—a notion savoring somewhat of the earlier conceptions prevalent in some quarters, of ante-natal influence—for Weismann[19] experimenting with *Vanessa* appears to have effected *color changes* by means of *temperature* and Tower[20] to have permanently upset that portion of the germ plasm of *Leptinotarsa* determining *pigmentation* by means of *humidity and temperature.* Thus, units may be made and unmade, and thus a foreign body or force entering a germ cell may conceivably cause a long series of reactions, each product becoming a new reagent affecting the determiners of many forms and tissues, if by chance lethal damage is not done before equilibrium is reached.

Moore in his paper "A Biochemical Conception of Dominance," says:

> When fertilization occurs, the germ cells bring into contact certain substances which are set free to react upon each other. Some of these

[19] "Germ Plasm," p. 379.

[20] "An Investigation in Chrysomelid Beetles of the Genus *Leptinotarsa.*"

substances may react simply with other substances and obey the Guldberg-Waage law of mass action, while others are of the nature of enzymes (ferments) and accelerate reactions which are already going forward at a very slow rate.[21]

It has been many times demonstrated that a positive determiner in a gamete of a simplex individual is not as "pure" as one from a duplex individual; furthermore, a soma developed from a zygote made up of a gamete containing a positive determiner, and another characterized by its absence, is not as strong in the character in question as one produced by two duplex parents. Thus, Davenport[22] has shown that in mating dominant white fowls with pigmented fowls there is often an "imperfection of dominance," giving rise to some more or less scattered pigmentation in F_1; this he demonstrated experimentally and, among other things, finds that

> Two white Leghorns crossed by a black Minorca produced only white hybrids, but the female hybrids at least had some black feathers. . . . No barring resulted from crossing white Leghorn with . . . black Minorca. . . . Of 26 hybrids between black Cochin and white Leghorn, 8 were barred black and white.

And he concludes that—

> alongside of dominance we must place an important modifying factor—the factor of the strength or potency of the representative of the given character in the germ plasm. This is clearly a variable quantity. If it is very potent we get a typically Mendelian result but if it is weak, we will have imperfect dominance or failure to develop altogether.

Thus the determiner for pigmentation in the black Cochin seems to be more concentrated than the same determiner in the black Minorca. Or is it possible that the antibody, although present in quantities theoretically in excess of the amount necessary for complete inhibition, fails to effect such inhibition completely for the same reason that the analogous phenomenon, due to some

[21] "A Biochemical Conception of Dominance," University of California Publications in Physiology, Vol. 4, No. 3, p. 11.

[22] "The Imperfection of Dominance," *American Breeders' Magazine*, Vol. 1, No. 1, p. 42.

mechanical necessity, is commonly observed in chemical experiments?

Obviously, the mass of the determiner for pigmentation is as potent a factor in determining the end result as the mass of the destroying antibody. The kind or quality of the pigment seems also to be a factor; the yellow or sorrel pigments seem to be destroyed more readily than the black or brown. It is also apparent that, due to a difference in the relative mass of the determiner and the antibody in the zygote, one cross may affect total destruction of the pigment while another parallel or reciprocal one may not. Thus, as above mentioned, Davenport's white Leghorn on black Minorca cross gave only white or nearly white offspring, while his parallel cross, viz., white Leghorn on black Cochin, gave considerable black pigment in the offspring. It has also been observed that the barred Plymouth Rock male, which is much less heavily pigmented than the female, when mated with a white Leghorn female gives only white offspring, but the reciprocal cross, viz., the white Leghorn male on the barred Plymouth Rock female gives barred, mottled, gray, creamy and white offspring regardless of sex. In this latter mating the two gametic elements, viz., the determiner for pigmentation and the destroying antibody, seem to be present in quite closely chemically balanced masses and it would be interesting to know whether in this cross the fluctuations across the color line are due to accidental variations in the strength of the individual gametic elements in question or to the Mendelian phenomenon.

There is still another white possessed by birds and mammals known as "structural white," characterizing some arctic animals such as the arctic fox, which is white the year around, and the arctic hare and the ptarmigan, which are pigmented at one season and white at the other. It would be interesting to know whether the fur and feathers of these animals in their unpigmented phases possess oxidized pigments. There are, more-

over white pea fowls. The gorgeous hues of the common pea fowl are due both to pigments and to defraction and it would be interesting to know whether the white pea fowl has lost its pigments or defraction surfaces, or both.

Animals of heavy pigmentation—as the blackbird, the crow and the negro—are said to be more subject to pre-senile and albinic white than others less heavily pigmented. Enzymes may be inhibited or destroyed by an excess of their own products. May it be indeed that the antibody (W) is itself a product of the determiner (N)?

To throw further light upon the whole problem, among other things, a careful study should be made of the behavior in inheritance of the age of graying of the hair and beard in man. If the conclusion of this paper presents a true picture, early graying of the hair and beard will be found to be dominant over the later manifestation of the same phenomenon. It is further anticipated that a chemical analysis of senile white and juvenile white tissues will show the same absence of somatic pigment as Gortner[23] has shown in his study of albinic and dominant whites.

In this study of Shorthorn cattle nine theoretical gametic coat-color types are defined. As previously stated, the striking fact is this: The roan of type No. 3 (which is reciprocally colored as compared with the ordinary color pattern of cattle) is *never* observed, and quite probably the red of type 2 is also missing. The reason is apparently as follows: The antibody inhibiting and destroying the determiner R (for red pigment) first attacks through mechanical and chemical necessities the determiner for coat pigment in the somatic areas of Set 1 (roughly—flank, heart girth, forehead) and progresses systematically through the areas of Set 2 (roughly—underline, barrel, legs and quarters, head and neck) according to the following scheme:

[23] "Spiegler's 'White Melanin' as related to Dominant or Recessive White," THE AMERICAN NATURALIST, Vol. XLIV, p. 501.

TABLE VIII

Class or Stage	Amount of Antibody Present	Gametic Formula for Areas of First Attack. (Set 1)	Gametic Formula for Areas of Later Attack. (Set 2)	Number of Inheritance Units in Entire Coat Pigmentation	Examples of Some Breeds of Cattle Representative of the Respective Resting Stages of the Whitening Process
1.	None or too little to start inhibition.	w_2P_2	w_2P_2	One.	Angus and solid black breeds generally.
2.	Enough to inhibit the determiner for pigmentation of the areas of Set 1.	W_2P_2	w_2P_2	Two or two groups.	Holstein and spotted breeds generally.
3.	Enough more to inhibit the determiner for pigmentation of the areas of Set 2.	W_2P_2	W_2P_2	One.	White park cattle of Britain.
4.	Enough more to start reaction and to destroy the determiner for pigmentation of the areas of Set 1.	w_2p_2	W_2P_2	Two or two groups.	Not represented by any breed nor ever observed in mongrels.
5.	Enough more to continue reaction and to destroy the determiner for pigmentation of the areas of Set 2.	w_2p_2	w_2p_2	One.	Occasional albinos.
6.	Enough more to deposit excess of antibody in place of determiner for pigmentation of areas of Set 1.	W_2p_2	w_2p_2	Two or two groups.	White Shorthorns of Type 9 of this paper.
7.	Enough more to deposit excess of antibody in place of determiner for pigmentation of areas of Set 2.	W_2p_2	W_2p_2	One.	Remotely possible that some strains of British white park cattle are of this type.

W = presence of antibody. P = presence of determiner for pigmentation.
w = absence of antibody. p = absence of determiner for pigmentation.

In Shorthorn cattle, classes 4, 5 and 7 of this table are not met with, neither are conditions parallel to class 4 ever observed in any other mammals. The further explanation may be as follows: Reaction between W and R does not begin until an excess of W is present (a condition not hard to parallel in the chemical laboratory) but when reaction does begin it is quite rapid, destroying all of R and most likely leaving an excess of W at the point of first attack. This would eliminate Class 4 (type 3 of the series previously described) and Class 5 (pure albinos) of this table. There may be "albino" cattle;

Pearson[24] reported a rumor of a herd of such but he was unable to locate it. Wilcox and Smith[25] describe a race of white cattle—Polled Albino—made by crossing a white Shorthorn cow with a polled bull of unknown breeding. The Swedish cattle were thought to possess "pink eyes" and if so were probably albinic in their entire coat; the Polled Albinos are doubtless "partial albinos." White Shorthorn cattle are generally blue-eyed, however, a considerable percentage are brown eyed.

The following chart of the ancestry and offspring of "White Rose," the first cow purchased by Mr. J. F. Hagaman, of Leonard, Mich., is prepared from data supplied by him:

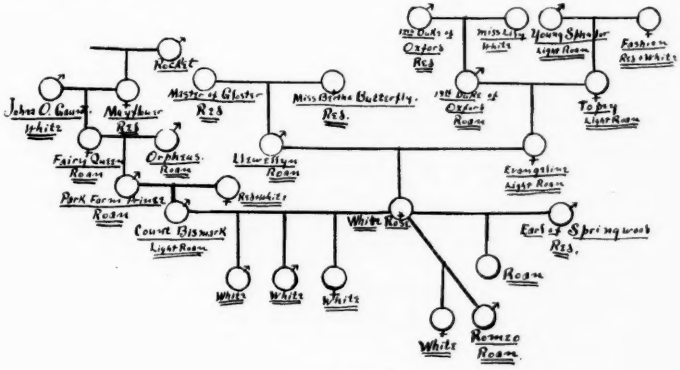

CHART NO. 3

He also writes:

I purchased another cow, Daisy Dean, red and white. All her ancestors were red, red and white, or roans. She was bred to Park Farm Prince (roan) and produced twin bull calves both *white*. They were exactly alike and were made into steers. A drover took them to Boston where they sold for $500. . . . All the white calves had *blue* eyes, *flesh-colored* noses and *light* skins.

Dr. D. M. Kipps, of Fort Royal, Va., writes:

I feel sure I never had a white Shorthorn with a *black* nose; I had one or two that had slightly cloudy noses. I think every one had pink

[24] "On the Inheritance of Coat-Colour in Cattle," *Biometrika*, 1905–06, p. 436.

[25] "Farmers' Cyclopedia of Live Stock," p. 369.

skin underlaying the white coat and *nearly* every one had slightly reddish hair on the inside and around the outer rim or auricle of the ear.

Mr. J. H. Hawkins, of Xenia, O., writes:

Will say I have never seen a white Shorthorn with pink eyes. My white Shorthorns have pure white coats, pink skins and brown eyes. As to black noses, they are not a rare thing to see . . . now and then.

Shorthorn cattle were made from the Anglo-Saxon reds —Class 1 of the above table No. VIII; the Flecking—Class 6; the Romano-British—Class 3, and probably some other primitive types. Evidently none of the breeds of domestic cattle has yet reached stage 7, *i. e.*, solid dominant white not capable of reversion. The Shorthorns of to-day present all the possible combinations of Classes 1, 2, 3 and 6.

In reference to the fact that the race of duplex yellow mice has never been produced and in view of what Castle[26] says,—viz., that the union between germ cells carrying only yellow pigment is doubtless affected, still all such germ cells from some cause are doomed to destruction, may it not be that in so delicately adjusted a mechanism two of these specific determiners present a lethal dose? May this not be one of the causes of the limits of hybridization and of the sterility of hybrids? The germ cells are doubtless distinguished by both a specific architectural and a specific chemical organization of the greatest nicety of adjustment and balance. The closest approach in the chemical world to their behavior is that of the enzymes, which, though not entering into reactions, may bring them about; while in the course of its own continuity the germ plasm gives rise to cells of its own kind, supplying them with bodies behaving in an enzyme-like manner sufficient for their own continuity and for a long series of ontogenetic processes.

It is obvious that a disturbance of some consequence would follow the advent of a foreign body or of unusual

[26] "Modified Mendelian Ratio among Yellow Mice," *Science*, December 16, 1910, Vol. XXXII, p. 868.

quantities of a normal body presented either by hybridizing or by osmotic intrusion; perhaps it may clarify the conception to make analogy to the degree and sequence of reactions in test-tubes or other containers of more complicated design holding the same chemical in varying quantities, places and degrees of nascency, wrought by the addition of varying quantities of the same reagent. The inhibitions and reactions expected from such conditions would begin at definite places, would continue in a more or less definite succession characteristic of each set of conditions, would complete a reaction first in definite parts and would proceed with varying degrees of speed, might effect a reaction and deposit an excess of reagent in some parts before even reaching other parts. Let there be an equilibrium following reaction; then add more of the reagent or of the chemical acted upon and it is easy to picture subsequent reactions all of which are closely analogous to the processes which the study of Shorthorn cattle leads us to believe have taken place within their gametes and zygotes. The behavior of their coat color and that of many other animals demand such behavior within the zygote. Thus such processes seem to account for the coarse mosaic or the spotted, and the fine mosaic or the roan color coat, the imperfection of dominance, reversion, the origin of the mottling and barring of fowls, the progressive dappling of horses, the peculiar behavior of "albino" guinea pigs, the characteristic behavior of coat pigment and patterns in Shorthorn cattle, and other similar phenomena. The stag, but not the doe, caribou possesses a beautiful white collar, and it may be that sex-limited characters are wrought by a sort of "havoc" or series of progressive reactions, preceding chemical equilibrium caused by the introduction of the essential sex-determiners.

A human family is recorded[27] in which a pre-senile gray spot occurs in the beard of the left cheek of many of its male members. In possible explanation, it is sug-

[27] Files Eugenics Record Office, Cold Spring Harbor, L. I.

gested that a small quantity of some antibody somehow inhibited or destroyed a portion of the determiner for pigmentation in the germ cell from which this family sprung. This indeed points toward a possibility that unit characters may arise from a partial destruction of larger units; that a determiner for a unit character behaving precisely in unit fashion may be a complex capable of being shattered into a large number of independently behaving characters. Small as the germ cell is and quantitatively insignificant as the determiner for the skin and hair pigment must be, the facts demand that this body consist of many molecules arranged in definite structure, each one destined for a somewhat definite ontogenetic process leading to a definite somatic end. Thus the often inherited specific color mark seems to indicate that a color pattern once produced—no matter how intricate or complex—will reproduce itself exactly until its determiners are disturbed by unbalanced bodies or forces presented by fertilization or otherwise.

The Shorthorns are a race of white cattle caught in the making and preserved in the nascent state by a rigid selection. It is thus conceivable that mutations may arise constantly, and that they may be progressive in character. Complications resulting in somatic effect are legion, but nothing occurs in the germ cell giving rise to new characters, splitting up and combining others and dropping out still others, that can not be analogously pictured with the simple operations of the chemical laboratory, and as Shull's[28] illuminating "Simple Chemical Device to Illustrate Mendelian Inheritance" seems to indicate, the analogy is too constant and too far-reaching to be cast aside as a mere pedagogical device. It may indeed be a simple statement of facts of intra-gametic and zygotic behavior and the analogy may no longer be needed to picture the actual conditions.

[28] *The Plant World,* Vol. 12, pp. 145–153, July, 1909, and companion paper, "The 'Presence and Absence' Hypothesis," THE AMERICAN NATURALIST, Vol. XLIII, No. 511, pp. 410–419, July, 1909.

The evidence of this study of Shorthorn cattle is to support that theory of unit segregation incompatible with a somatic blend in the *ultimate* unit, and that theory of heredity permitting intra-zygotic inhibition and reaction *in response to specific set conditions.*

The mutually corroborative evidence of the authentic history of this breed of cattle, the behavior of their coat pigments and patterns as recorded in the most extended authentic records of pedigree breeding of domestic animals, analogy to the occurrence and behavior of pigments in other animals, and the close fitting of the final working hypothesis, amply justify the following conclusions:

1. Shorthorn cattle as a race possess two kinds of white hair. (A) White, dominant to all pigments (analogous to the white of the Leghorn fowl) in a series of areas varying somewhat in size and shape but in a given individual always definite and genetically independent—a few at the front flank belt, a larger number or larger areas about the rear flank belt, a few along the underline and a fine network covering the remainder of the body. A few animals from their Romano-British ancestry have the entire coat of dominant white. An area of dominant white may be duplex or it may be simplex. In the former case its possessor will throw only gametes with determiners for dominant white; in the latter alternately gametes with determiners for dominant white and for red. (B) White, recessive to all pigments (analogous to the white of the Silkie fowl) in a series of definite areas generally smaller than those of the dominant white, forming a fine network about the neck and head, the sides and back, and the hind quarters and legs—quite precisely excluding the areas of the dominant white network. From their Dutch ancestry, this mosaic may in some strains be quite coarse. It is doubtful if a strain albinic white in its entire coat exists within the Shorthorn breed.

2. The color effect of an individual Shorthorn is determined by the registering of fortuitously one of the alternate color phases of each of the genetically independent

color areas gametically possessed by each of the two parents, together with such intra-zygotic inhibitions and reactions between the determiner for pigmentation (R) and the antibody (W) as may result from definite concentrations and intimacy of these two bodies presented by the two parents upon the formation of the zygote.

SUPPLEMENTARY OBSERVATIONS ON THE DEVELOPMENT OF THE CANADIAN OYSTER

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IN the AMERICAN NATURALIST of January, 1905, January, 1909, June, 1910, I have given some account of observations (in 1904) on the development of the oyster at Malpeque, Richmond Bay, Prince Edward Island, Canada.

Opportunity to verify, continue, and extend these observations was again afforded in 1909, when I studied the oyster in the most important centers along the east coast of New Brunswick.

In the present summer, 1911, being occupied at the Pacific Biological Station of Canada, in Departure Bay, near Nanaimo, Vancouver Island, I have the privilege of observing some of the Prince Edward Island oysters transplanted to this vicinity in 1905, as well as adding to my acquaintance the little British Columbia oyster, so different in size, appearance, habits and reproduction.

In the intermediate years, not being located in oyster regions, I devoted a good deal of time to other bivalve-larvæ, largely with a view to making my studies of the oyster more secure, the main results of which have been given in a paper "On the Recognition of Bivalve Larvæ in Plankton Collections," unreasonably delayed in publication at Ottawa.

In all this work I have kept sample preservations with dates and localities, which have often proved of great service in judging of questions that subsequently arose.

My first work began where that of Brooks left off, and showed for the first time that later stages of the oyster-larva undoubtedly exist, and when, where and how they

may be procured, as well as the length of the period of their free-swimming life. The larvæ obtained by Brooks, Rice, Ryder, Winslow, and others were obtained by culture from fertilized eggs, and were at most six days old, and in the young straight-hinge stage. In Europe larvæ of a similar age, size and structure had been taken from the infra-branchial cavity of the parent oyster by Davaine, Lacaze-Duthiers, Costé, De la Blanchère, Gwyn Jeffries, Saunders, Salensky, Möbius, Horst and Huxley, but the older, later or larger stages were quite unknown. This left room for some speculation as to the exact time, place and manner in which the succeeding stages should be found, as well as occasioned the prevalent mistake that the free larva settles down at this period to become a fixed spat. Brooks wrote. "All my attempts to get later stages than these failed . . . and I am therefore unable to describe the manner in which the swimming embryo becomes converted into the adult, but I hope that this gap will be filled, either by future observations of my own or by those of some other embryologist." In a similar way Jackson, at a later period, speaks of "a blank in the knowledge of the development of the oyster." This "gap" or "blank" is now completely filled. My studies prove that the larva continues to live as a larva in the sea-water about oyster-beds for two or three weeks longer, where it swims about, feeds, grows and changes in structure, and that it first settles down to become a sedentary spat, fixed to shells or other objects, at an age of three to four weeks from fertilization—the length of time depending to some extent on temperature, food, individuality or such causes. This information has been gained through the method of procuring oyster-larvæ from the waters of oyster-areas by means of a plankton-net, and connecting them in series with younger stages obtained by fertilization and culture and with older stages obtained by catching spat on glass, shells, etc., so as to make out the complete life-history.

The discovery that the hitherto unknown stages of the

oyster-larva can be conveniently obtained by a plankton-net carries with it the possibility of a practical application of inestimable value in the culture of oysters. From the time of the early Roman Empire it has been known that oyster-spat can sometimes be obtained on ropes, anchors, piles of wharves, stones, shells or other natural or artificial objects in the sea, and some sort of method of culture has long been in use in many countries. At times men have risen to exalted conceptions of the possibility of finding a practicable, safe and sure method of catching, retaining, and rearing the young spat. I quote Winslow to the effect that "Thousands of dollars would be annually saved by the Connecticut oystermen if they could determine, with even approximate accuracy, the date when the attachment of the young oyster would occur. Hundreds of thousands would be saved if they had any reliable method of determining the probabilities of the season." This is now possible.

It is well known that oyster or other shells dried and whitened in the sun form the very best oyster-collectors or cultch. To put these back into the water haphazard has often resulted solely in the loss of all the labor of preparation. In even a few days they may become covered with a slimy coating which reduces or largely destroys their efficiency. The point is to be able to determine with accuracy, for each season and for every locality, when oyster-larvæ are present in the water full-grown and ready to settle as spat, so as not to run the risk of losing adequate value for the laboriously prepared cultch. A man instructed and qualified in the method of taking plankton and in identifying oyster-larvæ can tell almost to a day when is the proper time to put out cultch so as to obtain an abundant and copious set of spat. It is not enough to know about the time, or to know the time for certain previous years, or to know the average time.

Three methods are open to the expert: (1) Examination of the genital organs of adult oysters to determine

when the eggs are ripe, (2) examination of the sea-water to learn if oyster-larvæ are present and in what stage, (3) examination of natural or improvised objects in the water to discover if young spat are already formed. The first is not immediately determinative because of the long period of development separating spawning and spatting. The last is not very practicable because of the difficulty of finding and recognizing the youngest spat before the period is gone by for putting out cultch. The second is the only practicable and conclusive method and its efficiency is proportionate to the number, care and accuracy of the observations. Its success will increase with experience.

This method makes use of the colossal number of larvæ lavishly provided by nature to offset the exigencies and accidents of life and insure a reasonable chance of keeping up the stock. I believe that all the larvæ an army of men could raise up and turn into the sea would not materially alter the number of successful individuals in the set of spat. But on the other hand a few culturists could enormously increase the chances for a successful catch by spreading an abundance of suitably prepared cultch at the proper time and place.

In the paper of 1909 I have described the method of obtaining plankton, the appearances and measurements of the oyster-larvæ to be recognized, the time of the year to begin making observations. In the paper on "Bivalve Larvæ" I distinguish in sizes, shapes, colors, the commonly occurring associates of the oyster-larvæ which might be taken for the latter. In the present paper, after long reflection, I suggest a practical application of the knowledge acquired.

I should not omit to mention that the paper of 1910 connects the larva, through the youngest microscopic spat, with the macroscopic spat of fishermen and finally with the adult. Similarly in 1909 I performed extensive artificial-fertilization experiments, while at Shediac, Caraquette and Malpeque, in order to connect the small-

est plankton stages of oyster-larvæ with culture-stages and through these back to the egg. Larvæ by the million were reared in beakers of sea-water at a temperature little above 20° C. and with a specific gravity (salinity) varying somewhat under 1020. I also carried Caraquette oysters to Malpeque and raised up larvæ from eggs cross-fertilized between two such obviously different varieties as the small, narrow, curved, thick, hard and heavy Caraquette oyster and the fine, large, broad, straight, clean, smooth specimens from the Curtain Island beds.

In 1896 and again in 1905 the Canadian Government had Atlantic oysters transshiped to the Pacific and put out at selected places. In the latter year some of the places were chosen by Captain Kemp, expert in oyster culture.

Being occupied this summer at our Pacific Biological Station, I have taken advantage (although not requested to do so) of my proximity to three of these places to search for the transplanted Prince Edward Island oysters, and to examine plankton taken in the vicinity. At the first place, Hammond Bay, being a small bay and close to hand, I could easily over-run all the beach at low water, and soon discovered the dead shells that had been deposited too far above low-water mark. At Nanoose Bay, some twelve miles away, perhaps five miles long and a mile and a half wide, with extensive flats at low tides, this was not so easily done. Having spent three summers with Captain Kemp, I thought now to test my judgment of where he would select to deposit the oysters. As the tide was unfavorable at my first visit I used the dredge, and was afterwards surprised to learn that I had actually calculated to within a few rods of the place. At the second visit I went to look at other parts of the bay, but on the third returned and, with a favorable tide, could wade and pick up some of the oysters. This was at 3 P. M., July 17, and I took 16 fine living specimens of the Malpeque oyster for examination—two or three of them with pieces of Prince Edward Island red-sandstone still

attached to them. They varied from two and three fourths to five inches in length, some of them showing considerable growth. This proves that Atlantic oysters can be transplanted to the Pacific and remain healthy and grow. Upon reaching home I proceeded to examine some of the oysters and it turned out that only one had already spawned while the other fifteen were ripe and generally somewhat distended with eggs or sperm. This proves that the transplanted oysters can come to maturity and ripen the reproductive elements.

At 7.10 P. M. of the same day I put together eggs and sperm in a tumbler of sea-water and at 7 A. M. next morning there was an abundance of segmentation stages and free-swimming larvæ. This proves that the oysters can spawn and that the eggs can develop into young. I make these statements because of a prevailing opinion that the transplanted oysters have all died, and the few people who think there are still some living are dogmatic in their assertion that they do not breed.

Plankton taken at intervals at Hammond and Nanoose Bays had not yielded any oyster larvæ, which became explainable upon finding the condition of the reproductive organs. A further observation on this was afforded on the 26th of July, when I examined a second lot (obtained at a very low tide the day before) from Nanoose Bay. The forty-seventh oyster examined was the first to yield good ripe eggs—all previous ones were spawned with the exception of four or five which were ripe males. The interval between these two visits had been the hottest of the summer and the oysters had nearly all spawned in this period—slightly later than is usual on the Atlantic. On the 27th I made a trip to Oyster Harbor (Ladysmith), about fifteen miles from here, where I had better luck in getting track of the few transplanted oysters. In a similar way I examined several individuals and took plankton which for the first time contained larvæ of the Atlantic oyster—recognizable by their shape and measurements but not presenting such a deep pink or brown

coloration as in their native home. For comparison with my former papers I will give the measurements of a single specimen with the characteristic postero-dorsal high umbos, the large convex left valve, and the smaller and flatter right valve, velum, foot, pigment spot and the rest. Ocular V, objective 4, 42 long by 37 high (=.289 × .255 mm.). This proves that larvæ grow up. There is only one other bit of evidence possible and that is to find spat. This I have not done as yet. It is too early for this year's spat and I have not seen any undoubted specimens of a former year's spat. One can judge that the comparatively few descendants of two and a half barrels deposited at Hammond Bay, five barrels at Nanoose Bay, and one barrel at Oyster Harbor, when dispersed over the broad areas at their command, would not prove very conspicuous objects, which is again complicated by the presence of millions of British Columbian oysters of varying sizes, shapes, and complexions.

I regard my findings as conclusive and would urge the transplanting of Atlantic oysters (*Ostrea virginica* Gmel.) to the Pacific in greater quantities. The Atlantic clam (*Mya arenaria* L.) has propagated enormously here notwithstanding the fact that it has more competitors in its particular habit than in its original home.

Ostrea lurida Carp.—Even before making any headway in the foregoing researches, I had begun to gather information on the occurrence, size, shape, color, structure, breeding, etc., of the British Columbia oyster.

This species is not common in Departure Bay, or in Hammond Bay, but a few specimens may be found under stones exposed at about one hour from low water in front of the C. P. R. cable house in the former, and just inside the far point of the latter, and are usually so broadly and solidly attached (with the left valve against the under side of the stone and hence uppermost) that it is scarcely possible to separate them without destroying

the attached surface. But on the extensive flats at the upper ends of Nanoose Bay and of Oyster Harbor they occur free on the surface by thousands and more or less covered with barnacles.

Good specimens reach two inches in length by an inch and a half in breadth, with a straight dorsal margin and a semicircular ventral curvature. The right, upper or smaller valve is nearly flat or but little convex and fits into the margins of the larger, convex, lower or left valve, the greater part of the lower and posterior margin being scalloped, while the left valve has corresponding ridges and points. The color is usually dark (those under stones lighter) with the older parts weathered grayish and the umbonal region of the left valve is often attached to a small stone or another oyster or bears a scar. Internally the shell is extensively pigmented, dark, with smaller bands or blotches of lighter pearl, while the muscle scar is rather lighter and banded. The mantle is broadly margined with dark, which may also creep up on to the abdomen.

The most interesting feature in connection with the Pacific oyster of Canada is its divergence in some respects from the mode of breeding of our Atlantic species. In the British Columbia form there is no primary separation of individuals into males and females—the sexes are united in each individual. In other words each individual is bisexual, monœcious or hermaphrodite. In this respect it is identical with the English or common European species (*Ostrea edulis* L.).

My first observations were made on July 12, on specimens procured under stones near the Biological Station. Nearly all appeared to be males, and, as they were of small size, I took it that, as commonly occurs, the males had ripened earliest. But one was of medium size and contained eggs that at once attracted my attention on account of their large size, opacity and rare exhibition of nucleus. Measured exactly as all my former measurements, these gave: Oc. V, obj. 2=6.5; Oc. V,

obj. 4 = 15; Oc. V, obj. 7 = 72. Another individual, obtained since, with an abundance of eggs oozing from the oviduct, pure and ripe, gave the almost unvarying measurement of the egg as: Oc. V, obj. 7 = 75. This when calculated is $75 \times 1.45\mu = 108.75\mu$ = slightly over .1 mm. = slightly over $\frac{1}{250}$ inch = fully twice the diameter of the egg of the Atlantic oyster, and perhaps identical in size with the egg of the English oyster.

In making measurements it is important to use only ripe eggs, as in this case, and to select those that are spherical or nearly so and not flattened by the weigth of the coverslip, as well as to extend the measurements to many individuals in order to exclude all possibility of a slip. The nucleus is between one half and two thirds the diameter of the egg.

Upon turning particularly to spermatozoa I found them in every individual—even between the eggs of those containing eggs in the gonad. The younger individuals had no ova, but all sperms. Some of the older ones had a few big, soft, opaque, irregular, elliptical, oval or nearly spherical eggs, scattered among irregular masses of less than half their size, which are balls of spermatids on the way to development into spermetozoa. One of these measured $46\mu \times 40\mu$, and each one is kept in a dancing or rolling movement, somewhat like that of many infusoria, by the flapping of the tails of the ripening sperms on the surface. Between these masses are millions of mature, free, dancing spermatozoa, of which the tails are rarely visible until one searches for them with a high power. I have not yet made extensive measurements of the sperm on account of the difficulty of measuring such exceedingly small objects with certainty, but I believe the sperm of the British Columbia oyster is smaller than that of the Prince Edward Island oyster, which may have some relation to the particular mode of fertilization, such as being introduced by the respiratory current. In some parts of the gonad ova may be plentiful, while at other parts there are only sperm-balls.

Later, in the warmer weather, the sperm may be pretty well run off and the reproductive organ contain mostly eggs. In this way the younger oysters, and the older oysters at the beginning of the season, may be physiologically males, while older oysters at the height of the breeding season may be physiologically females.

Oysters from Hammond Bay showed the same phenomena.

Upon finding an abundance of larger oysters on the surface at Nanoose Bay, I brought home a pail-full of picked specimens to serve as a convenient stock for observation and experiment. On July 16 I found a specimen with perhaps half a teaspoonful of eggs in various stages of segmentation, lying free in the lower valve—a mass of white granules. The ripe eggs ooze into the infra-branchial cavity and lie on and between the gills, *i. e.*, between the two folds of the mantle, where they are retained apparently without any retaining, sticky matrix. I suppose that it is here they first meet with ripe sperms from other individuals, for I do not believe that at this time the sperms of the same individual are physiologically capable. The whole oyster appears exhausted, the gills rent, the flesh collapsed, soft and parts of it almost rotten. On July 24 I opened one hundred of the stock supply and found six with eggs, embryos or conchiferous young, in the infra-branchial cavity. All the others were in process of spermogenesis and oogenesis.

An experiment that has often seemed possible to me is to do the same with the European oyster, by way of artificial fertilization, as Brooks did with the American oyster. Now that I had an oyster essentially the same as the European I tried it, and with seeming success, but of course it is difficult to be sure that sperm from another had not already had access to the eggs. Unripe eggs are no good; eggs already freed from the gonad may have come in contact with sperm. This restricts one to finding a specimen just before but just on the point of

extruding its eggs. I also tried Atlantic oyster eggs with Pacific oyster sperms, as well as Atlantic oyster sperms with Pacific oyster eggs, but without success, as one might suppose. I put eggs, embryos and larvæ of both species together under the same coverslip for comparison—those of the small British Columbia oyster looking like giants beside those of the large Prince Edward Island oyster. This is a curious phenomenon which I have several times observed on other species, *e. g.*, the very large eggs of Astarte compared with the small eggs of large species like Mactra.

For the study of segmentation, etc., the Atlantic species is of advantage on account of smaller size and greater transparency. The order of segmentation appears to be the same in both—both subject to variations such that it would require a great number of painstaking observations to decide exactly what is the normal mode in good healthy eggs. I have, on both sides of this continent, spent considerable time in trying to determine the order of segmentation, the cell-lineage, the planes of cleavage, the succession of nuclei, the effect of gravitation, the constant and continuous orientation of successive stages, the origin of the shell-gland and the mode of formation of the shell, etc., but can not discuss such subjects here. I may briefly state, however, that I believe Brooks failed to observe the shell-gland, in his original work, and at one particular stage mistook the relation of the shell-valves to the blastopore which made it necessary to reverse his orientation of the embryo—hence his use of the terms dorsal and ventral are misleading. The polar bodies are dorsal at first—later, if they persist, they may become displaced anteriorly. The blastopore is ventral, the velum anterior, the shell-gland dorsal, the mouth ventral. There is no foot, nor rudiment of it, in pre-conchiferous stages.

I have found conchiferous young of the British Columbia oyster retained within the parent's shell until their own minute shells were .138 mm. in length. I believe

they remain longer, for, according to Möbius, the young of the European oyster leaves the parent at a size of .15 to .18 mm. (Horst gives .16 mm.; Huxley $\frac{1}{150}$ inch). I have taken larvæ of *O. lurida* in plankton (identified by comparison with those from a parent, and also by the structure, shape and size) of a length of .165 mm. as well as different larger sizes. They still had a straight-hinge line of half the length of the shell—unlike the *O. virginica* which at this size is already passing into the umbo-stage and with a much shorter hinge-line. The larvæ of *O. lurida* are not pink or brown but have five or six dark blotches in the region of the liver and in the velum, in contrast to the general light shade of the rest of the animal.

THE EFFECTS OF ALCOHOL NOT INHERITED IN HYDATINA SENTA

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MANY experiments have been performed and much published concerning the effects of alcohol upon living organisms. Hodge, Calkins, Lieb, Woodruff, Estabrook, Matheny, and others, have observed its influence on the rate of growth and reproduction in certain unicelluar organisms. Abbott, Hodge and others have carried on some experiments with mammals by which they have demonstrated that the resistance to certain bacterial infections is lowered by the influence of alcohol. Hunt and Woodruff found an increase of susceptibility to certain poisons in the animals subjected to alcohol. Abel and Welch have summarized in general the pharmacological action and the pathological effects of alcohol upon man and some of the other mammals.[1]

Stockard has produced abnormal fish embryos and Féré has produced abnormal chick embryos by the use of alcohol, while Hodge, Newman, Sullivan and others have demonstrated the harmful influence of alcohol upon the embryos of mammals and man during pregnancy.

The evidence taken altogether with a few exceptions shows that when living organisms in any stage of their life are subjected to alcohol in appreciable quantities they are as a whole or in part unfavorably affected by it.

In nearly all of the previous work observations have been made especially upon the organisms themselves which have been directly subjected to the influence of alcohol at some stage of their life. As the harmful effects

[1] I am greatly indebted to Dr. F. E. Chidester for placing at my disposal his bibliography and notes of his forthcoming paper, "Cyclopia in Mammals."

of alcohol on the organisms subjected to its influence have been so conclusively demonstrated, it seems desirable to determine whether the offspring of alcoholic individuals in the subsequent generations are normal or show any of the weaknessess of their alcoholic ancestors. In other words the problem is to find out whether the descendants of alcoholic parents are in any way inferior to the normal individuals of the species and, if so, for how many generations the weakness continues.

That the parental use of alcohol in human beings affects some of the offspring in the first filial generation is undoubted by many observers, yet Pearson and Elderton have recently shown that the school children of alcoholic parents are as normal as the children of sober parents in physique and intelligence. However, the results set forth in this paper do not purport to have any relationship with the effects of alcohol upon man and his descendants.

While working with the rotifer, *Hydatina senta,* observations have been made which show that while alcohol decreases the rate of reproduction and increases the susceptibility to copper sulphate, still these harmful effects of alcohol disappear in the second generation after the alcohol has been removed from the culture water. The grandchildren show none of the alcoholic weaknessess of the grandparent, but are as normal as the individuals whose grandparents never were subjected to alcohol.

Hydatina senta can be readily reared and controlled in the laboratory in the manner described in a former paper. Alcohol can be added directly to the liquid medium in which the animals live. A large amount of the liquid is drawn through the mouth, indirectly by means of the pulsating bladder, into the alimentary canal, and the dialyzable parts pass through its walls into the body cavity and then finally out through the excretory ducts to the exterior of the body. In this way the animal is bathed both on the outside and on the inside of the body by the solution in which it is living. Consequently all internal parts and all

organs of the animal are subjected to whatever dialyzable chemical substance there may be in the solution.

The young females grow to maturity very rapidly and lay eggs which develop and hatch within a few hours. This extremely short life-cycle, from egg to egg in forty-eight hours, more or less, makes this animal a very favorable form with which to work. Many generations can be reared in a short time and as much information gained in a few weeks as it would require years to obtain from some of the other forms.

Experiments were first carried out to determine what influence a $\frac{1}{4}$ per cent., $\frac{1}{2}$ per cent. and 1 per cent. alcoholic solution had upon the race when it was subjected to it continuously for many successive generations. Precautions were taken to have all conditions, excluding the alcoholic conditions, in each generation exactly identical. The experiments were conducted in the same room so that the temperature was always uniform for each generation. The same amount of food culture from the same jar was always mixed with the same amount of water or with the same amounts of the various alcoholic solutions thus making the proportion of food culture to the mixture always alike. This mixture was then poured out into watch glasses and one young female rotifer put into each glass. At the end of forty-eight hours the young female had matured, layed eggs some of which had hatched, and young daughter-females would be found swimming in the dish. One of these daughter-females was isolated to start the next generation in the same manner as the mother was originally isolated. This was continued for twenty-eight consecutive generations. The twenty young females which were isolated to form the first generation were the grandchildren of the same grandmother, thus making the control, and the other three strains or groups all start originally from one female of one race. This was a very vigorous race hatched from a winter egg which was taken from a general mixed culture jar in the early spring.

Table I shows the detailed and summarized data of the

TABLE I

SHOWING THAT THE CONTINUOUS INFLUENCE OF ALCOHOL ON SUCCESSIVE GENERATIONS RETARDS THE RATE OF REPRODUCTION IN PROPORTION TO THE AMOUNT OF ALCOHOL USED

Time	Control			0.25 % Alcoholic Solution			0.5 % Alcoholic Solution			1 % Alcoholic Solution			Generation
	Young Females Isolated	Offspring Produced	Av. No. of Offspring	Young Females Isolated	Offspring Produced	Av. No. of Offspring	Young Females Isolated	Offspring Produced	Av. No. of Offspring	Young Females Isolated	Offspring Produced	Av. No. of Offspring	
M. May 3, 1911 .. / M. May 5, 1911 ..	5	25	5	5	28	5.37	5	31	6.2	5	22	4.4	I
Eve. May 5, 1911 .. / Eve. May 7, 1911 ..	5	30	6	5	30	6	5	20	4	5	12	2.4	II
Eve. May 7, 1911... / Eve. May 9, 1911 ..	4	22	5.5	5	31	6.2	4	23	5.75	5	9	1.8	III
Eve. May 9, 1911 .. / P.M. May 11, 1911 ..	4	24	6	4	26	6.5	5	22	4.4	5	3	0.6	IV
Eve. May 12, 1911 .. / Eve. May 14, 1911 ..	5	51	10.2	5	5	1	5	11	2.2	5	5	1	V
Eve. May 14, 1911... / Eve. May 16, 1911 ..	5	12	2.4	4	15	3.75	5	8	1.6	4	1	0.25	VI
Eve. May 16, 1911 .. / Eve. May 18, 1911 ..	5	47	9.4	5	22	4.4	5	19	3.8	1	0	0	VII
Eve. May 18, 1911 .. / Eve. May 20, 1911 ..	5	53	10.6	5	24	4.8	5	16	3.2	4	15	3.75	VIII
Eve. May 20, 1911 .. / Eve. May 22, 1911 ..	5	64	12.75	5	50	10	5	31	6.2	3	16	5.33	IX
Eve. May 22, 1911 .. / Eve. May 24, 1911 ..	5	25	5	5	25	5	5	12	2.4	5	5	1	X
Eve. May 24, 1911 .. / A.M. May 27, 1911 ..	5	72	14.4	5	68	13.6	4	40	10	4	15	3.75	XI
Eve. May 27, 1911 .. / Eve. May 29, 1911 ..	5	40	8	4	17	4.25	5	16	3.2	4	2	0.5	XII
Eve. May 29, 1911 .. / Eve. May 31, 1911 ..	5	35	7	5	12	2.4	5	10	2	4	4	1	XIII
Eve. May 31, 1911 .. / Eve. June 2, 1911 ..	5	31	6.2	5	26	5.2	5	9	1.8	5	14	2.8	XIV

TABLE I (*Continued*)

Time	Control			0.25% Alcoholic Solution			0.5% Alcoholic Solution			1% Alcoholic Solution			Generation
	Young Females Isolated	Offspring Produced	Av. No. of Offspring	Young Females Isolated	Offspring Produced	Av. No. of Offspring	Young Females Isolated	Offspring Produced	Av No of Offspring	Young Females Isolated	Offspring Produced	Av. No. of Offspring	
Eve. June 2, 1911 ..	5	30	6	5	13	2.6	5	7	1.4	4	5	1.25	XV
Eve. June 4, 1911 ..													
Eve. June 4, 1911 ..	5	43	8.6	1	0	0	3	11	3.66	5	13	2.6	XVI
Eve. June 7, 1911 ..													
Eve. June 7, 1911 ..	5	36	7.2	—	—	——	3	12	4	5	8	1.6	XVII
Eve. June 9, 1911 ..													
Eve. June 9, 1911 ..	5	57	11.4	1	3	3	5	18	3.6	5	2	0.4	XVIII
Eve. June 11, 1911 ..													
Eve. June 11, 1911 ..	4	26	6.5	5	6	1.2	5	5	1	4	2	0.5	XIX
Eve. June 13, 1911 ..													
Eve. June 14, 1911 ..	5	31	6.2	3	9	3	4	16	4	4	2	0.5	XX
Eve. June 16, 1911 ..													
Eve. June 16, 1911 ..	5	21	4.2	5	19	3.8	5	10	2	4	0	0	XXI
Eve. June 18, 1911 ..													
Eve. June 18, 1911 ..	5	91	18.2	5	38	7.6	5	20	4	5	5	1	XXII
Eve. June 21, 1911 ..													
A.M. June 22, 1911 ..	4	40	10	4	29	7.25	5	9	1.8	5	7	1.4	XXIII
A.M. June 24, 1911 ..													
A.M. June 24, 1911 ..	10	69	6.9	5	24	4.8	5	12	2.4	5	5	1	XXIV
A.M. June 26, 1911 ..													
A.M. June 26, 1911 ..	5	68	13.6	5	37	7.4	4	8	2	5	7	1.4	XXV
A.M. June 28, 1911 ..													
A.M. June 28, 1911 ..	5	79	15.8	5	14	2.8	5	8	1.6	3	1	0.33	XXVI
A.M. June 30, 1911 ..													
A.M. June 30, 1911 ..	5	52	10.4	1	4	4	5	20	4	5	4	0.8	XXVII
A.M. July 2, 1911 ..													
A.M. July 2, 1911 ..	5	15	3	3	1	0.33	1	0	0	3	3	1	XXVIII
A.M. July 4, 1911 ..													
Summary.....	141	1,189	8.43	115	576	5.00+	128	424	3.31	121	187	1.54+	

observations made upon the twenty-eight generations while they were subjected to the influence of the alcohol. One can not compare strictly the number of individuals in the different generations because of the changed conditions, especially of the temperature, and in some instances the length of time between the generations. However, the ratios between the strains or groups in all the generations may be compared and will show general uniformity. The first few generations of the $\frac{1}{4}$ per cent., and the $\frac{1}{2}$ per cent. alcoholic strains show a fluctuation in the rate of reproduction above and below that of the control; but this rate of reproduction in the $\frac{1}{4}$ per cent. alcoholic strain never rises to that of the control after the sixth generation. In the $\frac{1}{2}$ per cent. alcoholic strain the rate of reproduction never rises to that of the control after the third generation. In the 1 per cent. alcoholic strain the rate of reproduction even in the first generation does not equal that of the control. The summary shows in the average number of offspring for each female that the alcoholic strains differ in the rate of reproduction according to the amount of alcohol used. The more alcohol used the lower the rate of reproduction.

Another test to show the influence of the 1 per cent. alcohol in this same series was made by removing some of the individuals from the alcoholic solution and placing them in a 1/14,000 G. M. copper sulphate solution[2] and comparing the resisting power, or the ability to live, of this strain with that of the control when both were subjected to the copper sulphate solution. Table II shows the detailed data and Table III shows the summary. In the control 96.8 per cent. of the individuals lived forty-eight hours and produced young, while only 15 per cent. of the individuals taken from the 1 per cent. alcoholic strain in the XIII–XV generations lived forty-eight hours and produced young. This shows that the susceptibility to copper sulphate is greatly increased by the alcohol.

[2] Various solutions of copper sulphate were tried and the one employed was selected because it appeared to be of the maximum strength which the control could withstand.

TABLE II

SHOWING THE LOWER RESISTING POWER TO COPPER SULPHATE OF FEMALES REARED THIRTEEN TO FIFTEEN GENERATIONS IN A 1 PER CENT. ALCOHOLIC SOLUTION, AND ALSO SHOWING THAT THE RESISTING POWER HAS BEEN REESTABLISHED IN THE SECOND GENERATION AFTER THE ALCOHOL HAS BEEN REMOVED

(See Table III for Summary)

Experiment		Young Females Isolated	$\frac{1}{14000}$ G. M. Copper Sulphate Solution		
			24 Hours	36 Hours	48 Hours
1	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
	1 % alcohol	5	Alive	All dead	
2	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
	1 % alcohol	5	2 dead	All dead	
3	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
4	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
5	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
6	Control	5	Alive	Alive	Alive + young
	Second water generation	5	Alive	2 dead	3 alive + young
	1 % alcohol	5	Alive	4 dead	All dead
7	Control	5	Alive	Alive	Alive + young
	Second water generation	5	Alive	Alive	Alive + young
	1 % alcohol	5	Alive	4 dead	All dead
8	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
	1 % alcohol	5	3 dead	4 dead	All dead
9	Control	5	Alive	Alive + young	Alive + young
	Second water generation	5	Alive	Alive + young	Alive + young
	1 % alcohol	5	Alive	Alive but in poorer condition. Fewer young	1 dead. Others nearly dead Fewer young
10	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
	1 % alcohol	10	5 dead	5 dead, fewer young	6 dead + fewer young
11	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
	1 % alcohol	10	All dead		
12	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
	1 % alcohol	10	Alive	6 dead	6 dead + young
13	Control	10	Alive	Alive + young	Alive + young
	Second water generation	10	Alive	Alive + young	Alive + young
	1 % alcohol	10	3 dead	5 dead	7 dead + young
14	Control	20	4 dead	4 dead	4 dead + young
	Second water generation	20	3 dead	3 dead	3 dead + young
	1 % alcohol	20	19 dead	All dead	
15	Control	10	Alive	Alive	Alive + young
	Second water generation	10	6 dead	6 dead	6 dead, young
	1 % alcohol	10	All dead		

On July 1, these four strains of rotifers were carried to Woods Hole, Mass. Owing to the high temperature difficulty was experienced in growing proper food cultures and consequently by July 4 many of the animals had died and those that had survived were in a very bad condition and had very few offspring. It is interesting to note that more of the animals in the alcoholic strains died at this time in the twenty-eighth generation than in the control strain. The experiments were discontinued on account of these unfavorable conditions.

TABLE III

SHOWING SUMMARY OF TABLE II

	Copper Sulphate Solution					
	No. of Young Females Isolated	No. Died in 24 Hours	No. Died in 36 Hours	No. Died in 48 hours	No. Living at End of 48 Hours	Per Cent. Living at End of 48 Hours
Control	125	4	4	4	121	96.8
Second water generation ...	125	9	11	11	114	91.2
1 % alcohol....	100	52	78	85	15	15

The data in these three tables seem to show that alcohol from $\frac{1}{4}$ per cent. to 1 per cent. has a decided influence in lowering the rate of reproduction and also in lowering the power of resisting copper sulphate in the individuals of the 1 per cent. alcoholic strains. Presumably the resisting power to copper sulphate of the $\frac{1}{4}$ per cent. and the $\frac{1}{2}$ per cent. alcoholic strains was similarly lowered, but this was not determined.

This decrease of the reproduction rate and the increased susceptibility to copper sulphate can be considered as an indication that the "general vitality" of the race had been lowered in that the individuals were much inferior to the control individuals in their ability to cope with adverse conditions and to leave offspring with which to continue the race.

Since it is shown that alcohol decreases the "general vitality" of these animals the condition of their offspring now remains to be considered. Table IV gives the de-

tailed and summarized data of experiments showing the comparative reproduction rates of the offspring from the three alcoholic strains between generations XI and generation XXIII, special emphasis being laid upon the offspring from the parents in the 1 per cent. alcoholic strain, and the reproduction rate of the control or normal strain. Young females were isolated from the three alcoholic strains placed in media containing no alcohol and reared two generations parallel to the alcoholic strains. In this way they were under exactly the same conditions as the control strain. The isolations of young females from the ¼ per cent. and the ½ per cent. alcoholic strains were discontinued after a few experiments and the time devoted to experiments with the 1 per cent. strain. As this strain in Table I showed the lowest reproduction rate and was decidedly susceptible to the influence of copper sulphate, it was assumed to have suffered the most of any of the three strains subjected to alcohol and therefore was considered to be the most favorable to show the effects of alcohol upon the offspring. For the sake of clearness all the data are so arranged in Table IV as to show the three rates of reproduction in the same generation, of the control, alcoholic strains, and the alcoholic strain with the alcohol removed. In the first water generation the young females were isolated from the preceding alcoholic generation soon after hatching and reared in media containing no alcohol. Thus the formation of the egg from which each young female hatched and all the embryonic development occurred in the alcoholic solution. After being transferred to culture water lacking alcohol they grew to maturity and reproduced. This generation is called Water Generation I. Some of the young daughter-females from Water Generation I were isolated to form Water Generation II.

In comparing the rates of reproduction in the Water Generation I with the rate of reproduction in the same generation of the alcoholic strains it is seen that in all cases the rate of reproduction is higher in the Water

TABLE IV

SHOWING THAT THE NORMAL RATE OF REPRODUCTION IS RESTORED IN THE SECOND GENERATION AFTER THE ALCOHOL HAS BEEN REMOVED

Experiment	Time	Control			0.25 Per Cent. Alcohol						0.5 Per Cent. Alcohol						1 Per Cent. Alcohol						Alcoholic Generations	Water Generations
								Alcohol Removed. Water						Alcohol Removed. Water						Alcohol Removed. Water				
		Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring		
1	Eve. May 24, 1911	5	72	14.4	5	68	13.6	4	58	14.5	5	40	8	5	62	12.4	4	15	3.75	2	24	12	XI	I
	A.M. May 27, 1911																							
2	Eve. May 27, 1911	5	40	8	4	17	4.25	4	25	6.25	5	16	3.2	5	31	6.2	4	4	1	5	33	6.6	XII	I
	Eve. May 29, 1911																							
	Eve. May 29, 1911	5	35	7	5	12	2.4	5	35	7	5	10	2	5	41	8.2	4	4	1	5	35	7	XIII	II
	Eve. May 31, 1911																							
3	Eve. May 31, 1911	5	31	6.2	5	26	5.2	4	15	3.75	5	9	1.8	5	24	4.8	5	14	2.8	4	19	4.75	XIV	I
	Eve. June 2, 1911																							
	Eve. June 2, 1911	5	30	6	5	13	2.6	5	32	6.4	5	7	1.4	4	25	6.25	4	5	1.25	4	25	6.25	XV	II
	Eve. June 4, 1911																							
4	Eve. June 4, 1911	5	43	8.6	1	0	0	4	14	3.5	3	11	3.66	3	18	6	5	13	2.6	4	24	6	XVI	I
	Eve. June 7, 1911																							
	Eve. June 7, 1911	5	36	7.2	—	—	—	5	38	7.6	3	12	4	5	44	8.8	5	8	1.6	5	44	8.8	XVII	II
	Eve. June 9, 1911																							
5	Eve. June 9, 1911	5	57	11.4	1	3	3	—	—	—	5	18	3.6	5	12	2.4	5	2	0.4	5	12	2.4	XVIII	I
	Eve. June 11, 1911																							
	Eve. June 11, 1911	4	26	6.5	5	6	1.2	—	—	—	5	5	1	4	24	6	4	2	0.5	4	25	6.25	XIX	II
	Eve. June 13, 1911																							

TABLE IV (*Continued*)

Experiment	Time	Control			0.25 Per Cent. Alcohol						0.5 Per Cent. Alcohol						1 Per Cent Alcohol						Alcoholic Generations	Water Generations
		Young Females Isolated	Offspring Produced	Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Alcohol Removed. Water: Young Females Isolated	Alcohol Removed. Water: Offspring Produced	Alcohol Removed. Water: Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Alcohol Removed. Water: Young Females Isolated	Alcohol Removed. Water: Offspring Produced	Alcohol Removed. Water: Average No. of Offspring	Young Females Isolated	Offspring Produced	Average No. of Offspring	Alcohol Removed. Water: Young Females Isolated	Alcohol Removed. Water: Offspring Produced	Alcohol Removed. Water: Average No. of Offspring		
6	Eve. June 14, 1911	5	31	6.2	3	9	3	—	—	—	4	16	4	—	—	—	4	2	0.5	21	26	1.23	XX	I
	Eve. June 16, 1911																							
	Eve. June 16, 1911	5	21	4.2	5	19	3.8	—	—	—	5	10	2	—	—	—	4	0	0	29	126	4.34	XXI	II
	Eve. June 18, 1911																							
7	Eve. June 18, 1911	5	91	18.2	5	38	7.6	—	—	—	5	20	4	—	—	—	5	5	1	2	14	7	XXII	I
	Eve. June 21, 1911																							
	A.M. June 22, 1911	5	51	10.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9	90	10	XXIII	II
	Eve. June 23, 1911																							
	A.M. June 22, 1911	5	80	16	4	29	7.25	—	—	—	5	9	1.8	—	—	—	5	7	1.4	7	105	15	XXIII	II
	A.M. June 24, 1911																							
8	A.M. June 22, 1911	4	40	10	4	29	7.25	—	—	—	5	9	1.8	—	—	—	5	7	1.4	13	34	2.61	XXIII	I
	A.M. June 24, 1911																							
	A.M. June 24, 1911	10	69	6.9	5	2	24.8	—	—	—	5	12	2.4	—	—	—	5	5	1	29	258	8.89	XXIV	II
	A.M. June 26, 1911																							
	Summary	39	405	10.38	28	190	6.78	16	112	7	32	139	4.34	23	147	6.39	37	62	1.67	56	186	3.32	XI, XII, XIV, XVI, XVIII, XX, XXII, & XXIII.	I
	Summary	44	348	7.90	29	103	3.55	15	105	7	33	65	1.96	18	134	7.44	31	31	1	92	708	7.69	XIII, XV, XVII, XIX, XXI, XXII, XXIII & XXII.	II

Generation I than it is in any of the corresponding alcoholic strains. However, it never reaches that of the control. In the Water Generation II the rate of reproduction is much higher than it is in Water Generation I and *equals* the reproductive rate of the control. This demonstrates that one of the ill effects of alcohol is partially eliminated in the first generation and is entirely eliminated in the second generation after the alcohol has been removed.

Very probably the rate of reproduction of Water Generation I, which is lower than that of the control, is due to the fact that the females in their embryonic development were subjected to the influence of alcohol from which they never fully recovered after they were transferred to water solutions containing no alcohol. They were thus perhaps influenced while in the growth and maturation stages of the egg inside the body of the mother or, after the egg was laid in the alcoholic solution, in the embryonic stages which occurred inside the egg membrane before hatching. Soon after hatching the females were put into the solutions free from alcohol.

This transmission of a low reproduction rate to Water Generation I is in reality not a hereditary transmission of a characteristic, but is probably the result of the direct influence of the alcohol upon the mother during her embryonic development.

Tables II and III show the effect of copper sulphate upon Water Generation II. Of the individuals tested 91.2 per cent. lived forty-eight hours and produced young in the copper solution. This is only about 5 per cent. less than the number of individuals of the control living and producing young for the same length of time in the copper solution. It can be concluded from these observations that the resisting power to copper sulphate has been restored practically to normal and that these individuals are no more susceptible to its influence than the individuals of the control.

Billings states to the Committee of Fifty in his report,

which is based on over five thousand reports of cases of insanity, that: "Inherited tendency to insanity, due to the use of liquor by parents, is reported in one hundred and twenty-two cases . . . while six cases were ascribed to the intemperance of the grandparents. These statistics must be received with caution as showing possibilities rather than as definite evidence. To prove that the insanity of one generation is due to alcoholic excess of a previous generation, and is not merely a coincidence, requires that other causes of degeneration shall be carefully studied, and duly allowed for."

It is, however, evident from the six cases reported that some, at least, of the medical examiners believe in the transmission of alcoholic weaknessess from grandparents to grandchildren.

Bunge, from an investigation extending over two thousand families, found that chronic alcoholic poisoning in the father was the chief cause of the daughter's inability to suckle and that this inability was not usually recovered from in subsequent generations. These results have been severely criticized by Bluhm and their validity questioned.

Mariet and Cambemale gave considerable quantities of alcohol to a female dog during the last week of her pregnancy. She gave birth to a litter of seven puppies, of which four were dead, two apparently healthy but mentally backward, and one, No. 7, both physically and mentally backward. No. 7 was a female and grew to maturity free from the influence of alcohol and mated with an apparently healthy dog. All of the puppies of her first litter were abnormal to such a degree that they were considered worthless. One had club feet and a clefted palate, another had a conspicuous ductus Botalli, and another developed muscular atrophy in its hind legs.

If these observations and interpretations are correct they may demonstrate either the same fact that is shown in Water Generation I of Table IV, namely, that when a mother is subjected to the influence of alcohol during her

own embryonic development she shows some sign of weakness at her period of reproduction, or, that the grandchildren are affected by the influence of alcohol upon their grandparents. However, only one experiment alone like the above is not sufficient to prove anything, and furthermore, Hodge in speaking of dogs says: "We do not attach much importance to the greater percentage of deformity, since this is of somewhat common occurrence in kennels."

If the transmission of an alcoholic weakness to subsequent generations is possible in any living organism, it ought to be actually demonstrated in some manner, but if it is a delusion, the sooner it is dispelled the better. These experiments with *Hydatina senta* are an attempt to determine, in one race of animals only, whether certain alcoholic weaknesses are truly hereditary and the evidence found is negative.

It by no means follows that these results would be found to be true in man. Alcohol primarily affects the nervous system and may have a very different action on the highly organized nervous system of man than it does on the lowly organized *Hydatina,* whose nervous system is extremely simple. Furthermore, the germ substance in man is probably very different from the germ substance in the rotifer and alcohol might have a very different effect upon it.

SUMMARY

1. Four strains of parthenogenetic rotifers originally descended from the same female were observed throughout twenty-eight successive generations. One strain was kept as a control and the other three strains were kept in a $\frac{1}{4}$ per cent., a $\frac{1}{2}$ per cent. and a 1 per cent. solution of alcohol. The rate of reproduction was lower in the alcoholic strains than in the control and it was proportionally lowered according to the amount of alcohol used.

2. The individuals of the 1 per cent. alcoholic strain in the XI–XV generations showed a decidedly increased susceptibility to copper sulphate.

3. When the alcohol was removed in generations XI–XXII, the rate of reproduction increased noticeably in the first generation and in the second generation the reproduction rate equaled that of the control.

4. Individuals of the second generation after the alcohol had been removed were no more susceptible to copper sulphate than individuals which had never been subjected to alcohol.

5. The general conclusion is that alcohol in $\frac{1}{4}$ per cent., $\frac{1}{2}$ per cent., and 1 per cent. solutions is detrimental to this race of rotifers when it is subjected to it continuously for many generations. The weaknesses developed by the parental use of alcohol are partially eliminated in the first generation after the alcohol has been removed, and practically completely eliminated at the end of the second generation after the alcohol has been removed. In other words, the grandchildren possess none of the defects caused by alcohol in the grandparents.

6. These results in general show that alcohol in the percentages used affects only the somatic tissues of the animal, and if they are subjected to its influences indefinitely, generation after generation, the race would probably become extinct because of its "lowered resistance power" to unfavorable conditions. However, if the alcohol is removed it is possible for the race to recover and to regain its normal condition in two generations, thus showing that the germ substance is not permanently affected by the alcohol.

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